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List of supplemental items

Movie S1: PTEN $^{-/-}$ adult retina explant expressing PLAP (control), Armcx1 or Armcx1ΔTM. Related to figure 2.

Movie S2: E18 cortical neuron transfected with MitoDsRed and either GFP (control) or Armcx1F2AGFP. Related to figure 3.

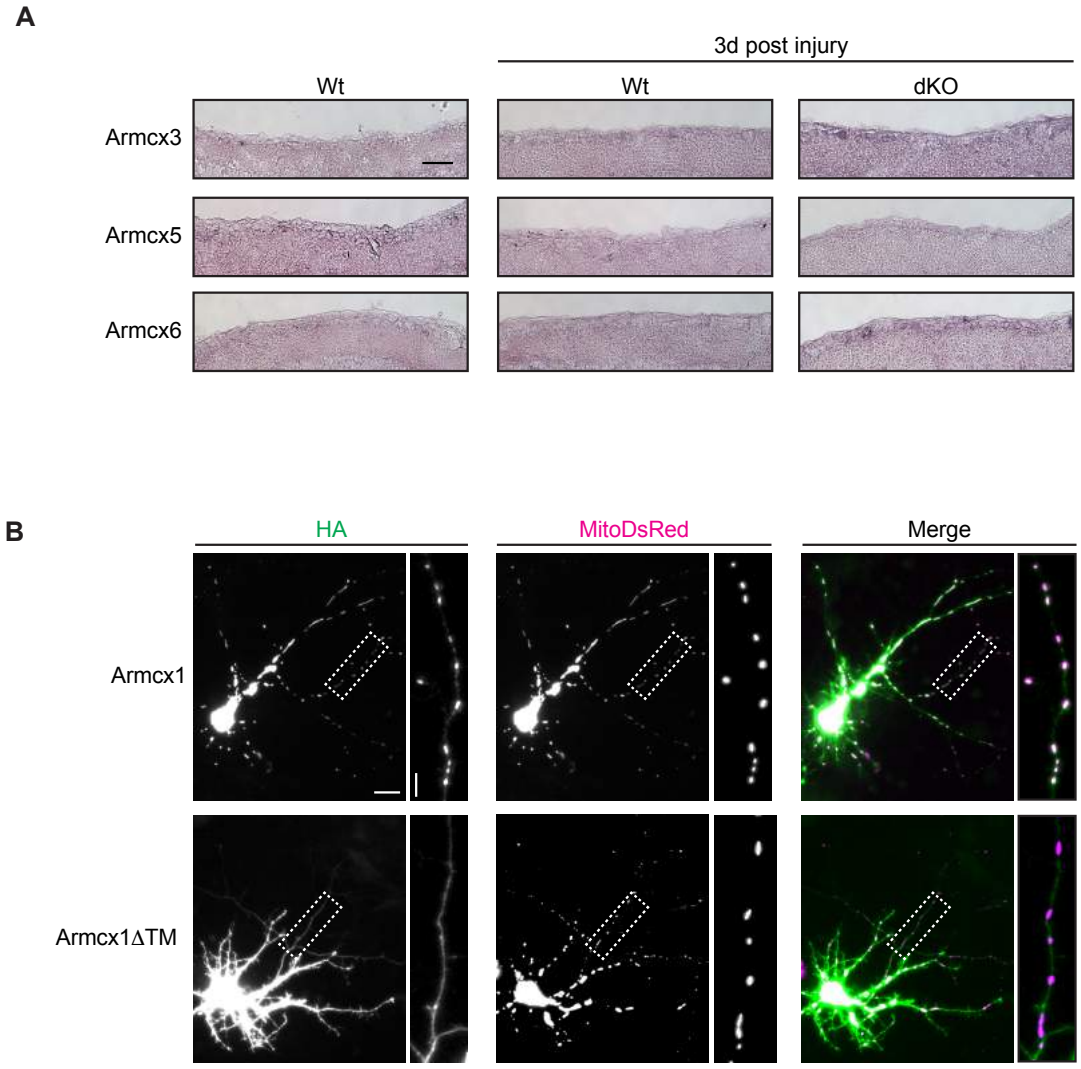

Figure S1. Expression of Armcx1 homologues in high regeneration capacities RGCs and Armcx1 co-localization with mitochondria.
Related to figure 1.
(A) *In situ* hybridization showing the mRNA levels of selected members of the Armcx cluster in mouse retina cross-sections of Wt and high regeneration mutant PTEN, SOCS3 double KO +CNTF (dKO) in intact conditions or 3 days post optic nerve crush. Scale bar=50µm. (B) Immunohistochemistry using anti-HA antibody of mouse cortical neurons co-transfected with MitoDsRed2 and either Armcx1-HA full length or Armcx1ΔTM-HA. Scale bar=20µm for the low magnification images and 5µm for the high magnification images.

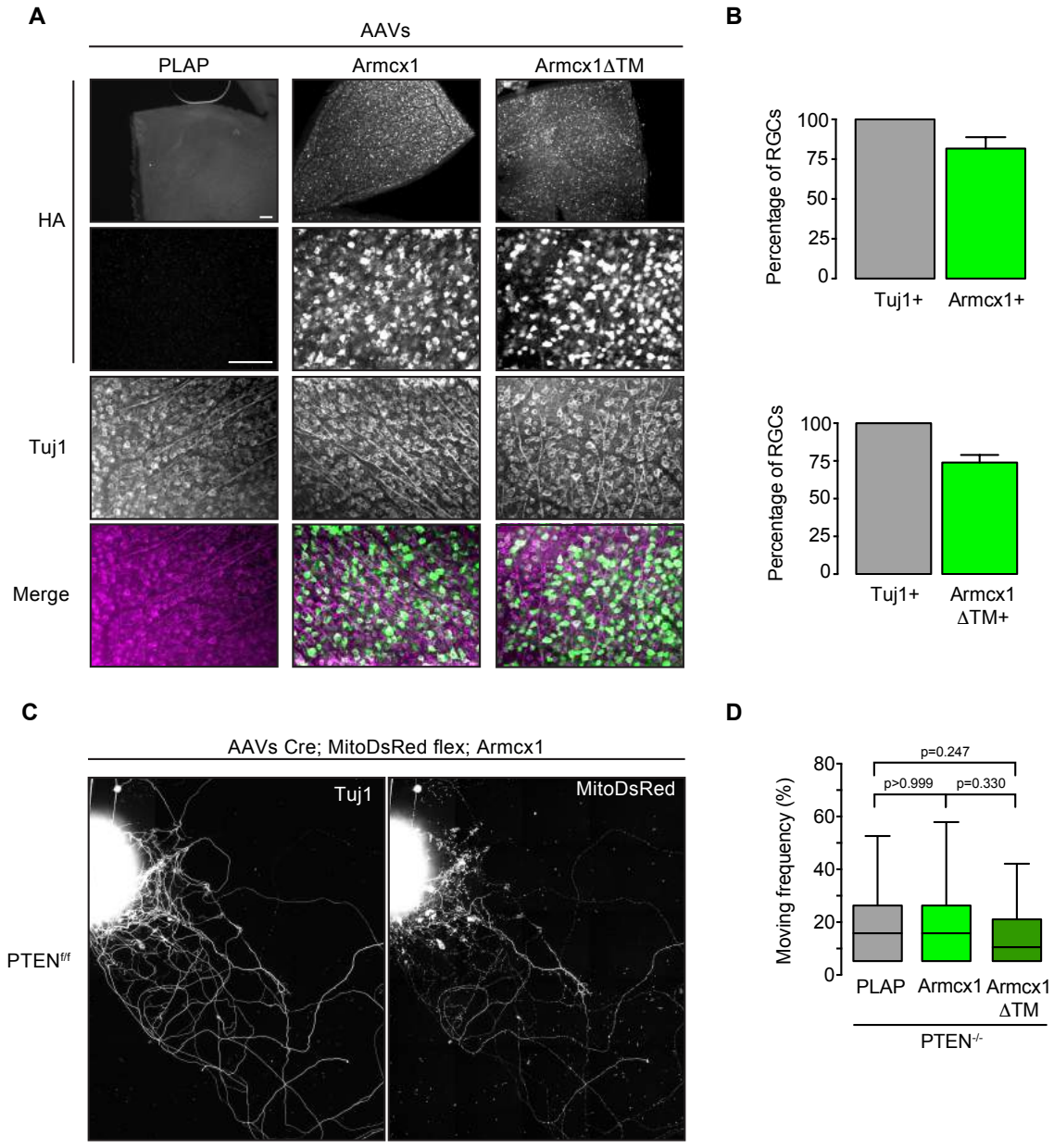

Figure S2. Characterization of AAV infected adult retina explants.
Related to Figure 2.

(A) Representative images of whole mount retinas from Wt mice injected with the indicated AAVs. 15 days post viral injections, mice were euthanized and immunohistochemistry using indicated antibodies was performed. Scale bar=100µm. (B) Quantification of the percentage of RGCs infected with AAV-Armcx1-HA *(upper)* and Armcx1ΔTM-HA *(lower)*. n=1632-2331 RGCs from 2 retina per conditions. (C) Adult retina explant culture of $PTEN^{f/f}$ mice co-injected with indicated AAVs. Explants were immunostained with Tuj1 antibody. The majority of Cre infected RGCs axons were also infected with MitoDsRed Flex Switch. Scale bar=100µm. (D) Box plot showing the moving frequency of motile mitochondria in axons from adult retina explants of $PTEN^{f/f}$ mice injected with the indicated AAVs. The horizontal line indicates the median and the whiskers the maximum and minimum of the distribution. n=221-348 mitochondria from 11-16 axons, from 3 independent experiments. Kruskal-Wallis test with Dunn's multiple comparisons test.

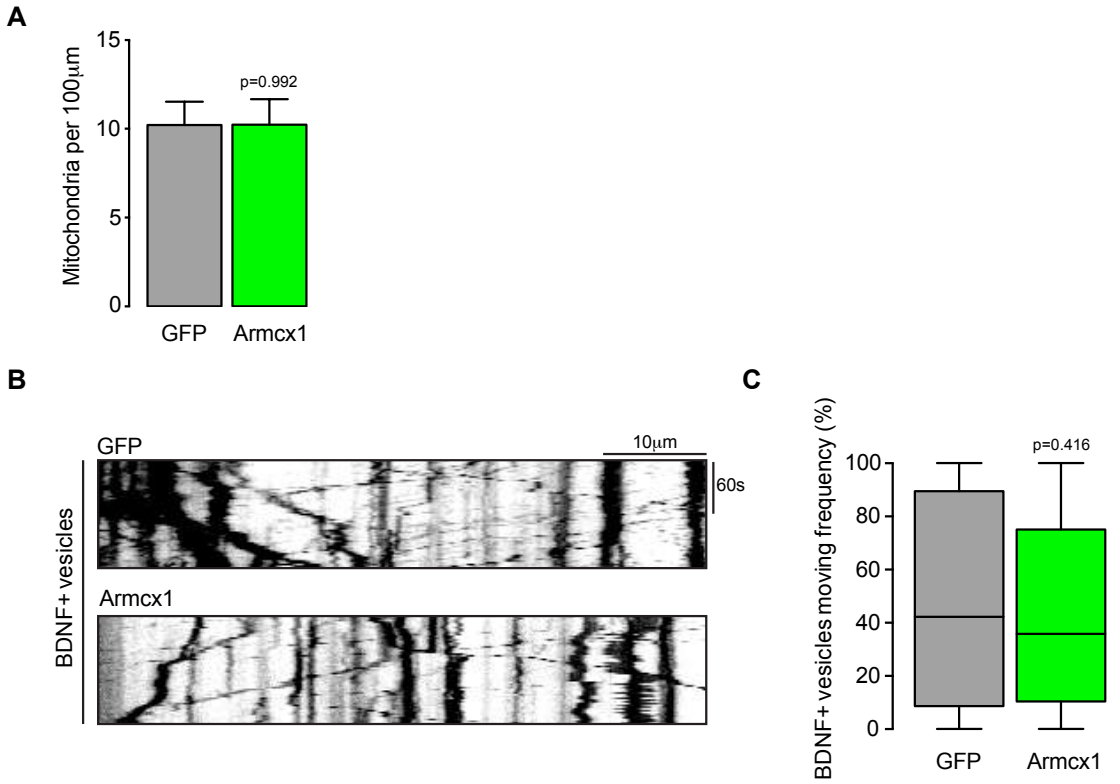

Figure S3. Armcx1 overexpression does not affect mitochondrial density and transport of BDNF positive vesicles.
Related to figure 3.
(A) Quantification of the mitochondrial density in axons of E18 cortical neurons co-transfected with MitoDsRed and either GFP or Armcx1-F2A-GFP. n=8-10 axons from 3 independent experiments. Two tailed Student's Unpaired *t*-test. (B) Representative kymographs from live imaging experiments of BDNF positive vesicles in E18 cortical neurons co-transfected with BDNF-RFP and either GFP or Armcx1-F2A-GFP. (C) Box plot showing the moving frequency of BDNF positive vesicles in axons from cortical neurons of the indicated genotypes. The horizontal line shows the median of the distribution. n=127-142 vesicles from 9 axons, 3 independent experiments. Mann-Whitney *U* test.

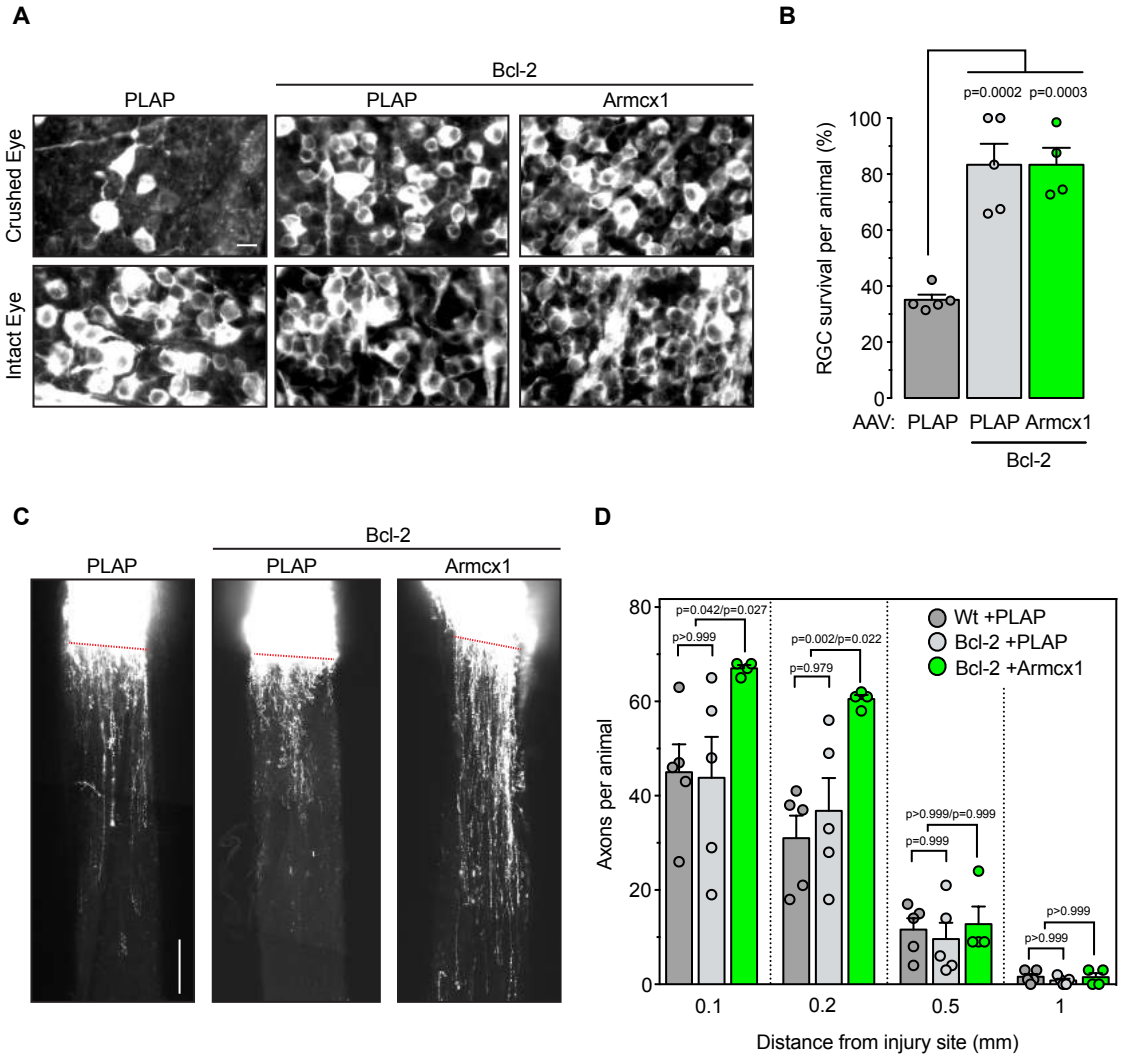

Figure S4. Armcx1 improve axonal regeneration independently of its survival effect. Related to figure 4.

(A) Tuj1 immunohistochemistry on whole mount retina from mice of the indicated genotypes. Retina from the crushed eye and intact eye of the same animal is shown. Scale bar=40µm. (B) Average percentage of RGC survival as measured by Tuj1 staining. Each dot represents one animal. n=4-5 animals per condition. One-way ANOVA, Tukey's multiple comparison test. (C) Optical sections (approximately 14µm) from whole mount cleared optic nerve collected 15 days post optic nerve crush (dashed red line) from Wt mice injected with AAV -PLAP, and Bcl2 mice injected with either AAV-PLAP or AAV-Armcx1. Axons were labeled with intraocular CTB injection. Scale bar=100µm. (D) Quantification of the average total number of axons growing past the injury site based on the z projection of the whole mount cleared optic nerve. Each dot represents one animal. n=4-5 animals per condition. One-way ANOVA, Holm-Sidak's multiple comparison test.

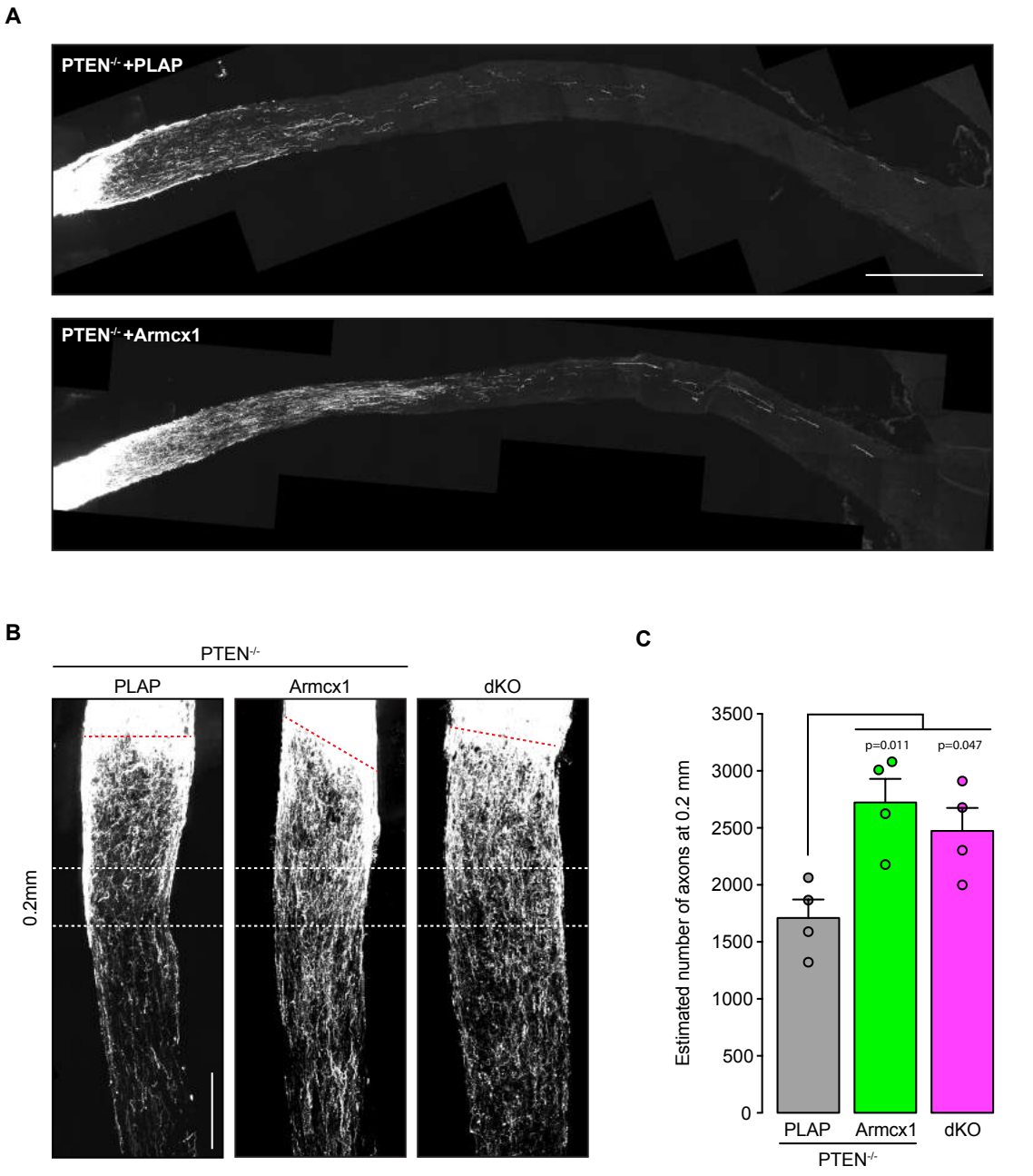

Figure S5. Armcx1 overexpression in PTEN$^{-/-}$ potentiates regeneration phenotype and partially recapitulates axonal regeneration phenotype of dKO.
Related to Figure 5.
(A) Optic nerve sections of PTEN$^{f/f}$ mice co-injected with AAV Cre and the indicated AAVs, 15 days post optic nerve crush. Axons were labeled with CTB injection. Scale bar=500µm. (B) Side by side comparison of optic nerve sections of PTEN$^{-/-}$ mice injected with the indicated AAVs and dKO, 15 days post optic nerve crush (dashed red line). All AAVs were injected and incubated in parallel in all conditions. Axons were labeled with intraocular CTB injection. Scale bar=100µm. (C) Bar plot showing the average estimated number of axons at 0.2mm from the injury site. Each dot represents one animal (2-6 cryo-sections per animal). n=4 animals per condition. One-way ANOVA, Tuckey's multiple comparison test.

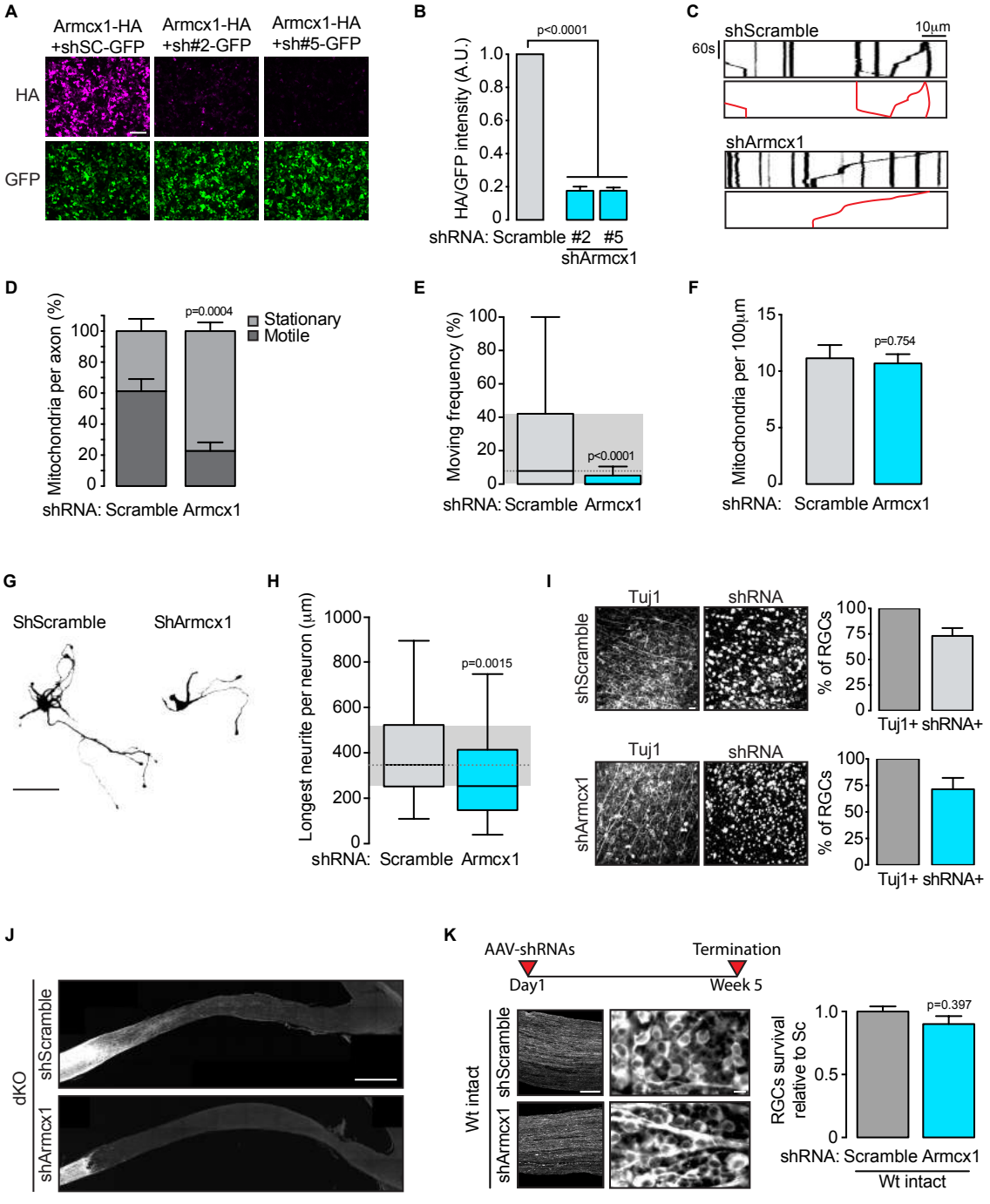

Figure S6. Armcx1 is necessary for dKO high regeneration phenotype.
Related to Figure 6

(A) Immunohistochemistry using the indicated antibody of HEK cells co-transfected with Armcx1-HA and either shRNA-Scramble-GFP, shRNA-Armcx1 #2, shRNA-Armcx1 #5.
Scale bar=100µm (B) Quantification of the intensity ratio from HA and GFP antibody signal. n=5 per conditions from 2 independent experiments with 2-3 technical replicates. One-way ANOVA, Tukey's multiple comparison test. (C) Representative kymographs from live imaging of mitochondria in E18 cortical neurons co-transfected with MitoDsRed and either shRNA Scramble or shRNA Armcx1. (D) Percentage of motile and stationary mitochondria per axon of E18 cortical neurons transfected as in C.

n=12-14 axons from 3 independent experiments. Two tailed Student's Unpaired *t*-test on the number of axons. (E) Box plot showing the moving frequency of mitochondria in axons from cortical neurons transfected as in C. n=142-149 mitochondria from 3 independent experiments (12-14 axons). Mann-Whitney *U* test. (F) Quantification of the mitochondrial density in axons of E18 cortical neurons co-transfected with either shScramble or shArmcx1. n=12-14 axons from 3 independent experiments. Two tailed Student's Unpaired *t*-test (G) Representative images of E18 cortical neurons transfected with shRNA Scramble or shRNA Armcx1 using anti GFP and RFP antibody, respectively. Scale bar=100µm. (H) Box plots showing the distribution of the measurements of the longest neurites in E18 cortical neurons transfected either with shRNA Scramble or shRNA Armcx1. n= 57 neurons per conditions from 3 independent experiments. Mann-Whitney *U* test. (I) Representative images and quantification of immunohistochemistry on whole mount retinas from Wt mice injected with either shRNA Scramble (upper) or shRNA Armcx1 (lower). Tissues were stained with antibody against Tuj1, GFP (shRNA Scramble) or RFP (shRNA Armcx1). Scale bar=80µm. (J) Optic nerve sections of dKO mice injected with the indicated AAVs, 15 days post optic nerve crush. Axons were labeled with CTB injection. Scale bar 500µm. (K) Experimental time line and *(left)* CTB-traced optic nerve sections (first column) and Tuj1 immunostaining of whole mount retina (second column) of Wt mice intra-vitreally injected with either AAV-shScramble or AAV-shArmcx1. Mice were euthanized 5 weeks post injection. Scale bar=100µm and 40µm, respectively. *(Right)* Quantification of RGC survival of mice with the indicated treatment. n=4-5 mice per condition. Mann-Whitney *U* test.

Movie S1. PTEN $^{-/-}$ adult retina explant expressing PLAP (control), Armcx1 or Armcx1ΔTM. Mitochondria were labeled using TMRM. Related to figure 2.

Movie S2. E18 cortical neuron transfected with MitoDsRed and either GFP (control) or Armcx1F2AGFP. Related to figure 3.